BRIEF REPORT

N-Acetylcysteine Reduces Extinction Responding and Induces Enduring Reductions in Cue- and Heroin-Induced Drug-Seeking

Wenhua Zhou and Peter W. Kalivas

Background: Previous studies show that the acute administration of N-acetylcysteine (NAC) inhibits the desire for cocaine in addicts and cocaine-seeking in animals.

Methods: Rats were trained to self-administer heroin, and the reinstatement model of drug seeking was used to determine whether chronic NAC treatment inhibited heroin-seeking.

Results: Daily NAC administration inhibited cue- and heroin-induced seeking. Moreover, repeated NAC administration during extinction training reduced extinction-responding and inhibited cue- and heroin-induced reinstatement for up to 40 days after discontinuing daily NAC injection.

Conclusions: These data show that daily NAC inhibits heroin-induced reinstatement and produces an enduring reduction in cue- and heroin-induced drug seeking for over 1 month after the last injection of NAC. Both the inhibitory effect of NAC on the reinstatement of heroin-seeking and the ability of NAC to reduce extinction-responding support clinical evaluation of repeated NAC administration to decrease in drug-seeking in heroin addicts.

Key Words: Addiction, extinction, heroin, N-acetylcysteine, reinstatement, self-administration

Relapse to drug-seeking behavior is a primary manifestation of drug addiction, and reducing relapse is a clinical index of a successful intervention (1). Relapse is modeled in rodents by measuring the reinstatement of drug-seeking behavior in animals that have undergone extinction training (2). Drug-seeking can be induced by stimuli akin to those eliciting relapse in addicts, such as presentation of drug-associated cues, stress, or a single dose of the drug itself.

Employing rats trained to self-administer cocaine, researchers have shown that acute pretreatment with the cysteine prodrug, N-acetylcysteine (NAC), prevents cocaine-induced reinstatement (3,4). This preclinical finding was recently translated into a small double blind clinical trial where NAC successfully reduced the desire for cocaine elicited by presenting cocaine-associated cues (5). N-acetylcysteine is thought to reduce reinstatement by restoring cystine–glutamate exchange, which brings the cocaine-reduced extracellular concentration of glutamate into the normal physiological range (4–5 μmol/L). The normalization of extracellular glutamate restores tone onto presynaptic metabotropic glutamate autoreceptors. The elevated tone on presynaptic inhibitory autoreceptors in the nucleus accumbens by NAC blunts the increased glutamate release associated with the reinstatement of drug-seeking (3,4).

The present study had two goals: 1) to determine whether, akin to cocaine, NAC pretreatment would inhibit cue- or heroin-induced reinstatement in rats trained to self-administer heroin; and 2) to determine whether daily administration of NAC could elicit an enduring reduction in cue and heroin reinstatement.

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Methods and Materials

Detailed methods are provided in Supplement 1. Male Sprague-Dawley rats were housed individually on a reversed 12-hour/12-hour light-dark cycle. All experiments were conducted during the dark period according to specifications of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were anesthetized with ketamine hydrochloric acid (87.5 mg/kg, IP) and xylazine (5 mg/kg, IP) and implanted with indwelling jugular catheters as described previously (6). Heroin self-administration training was conducted during 3-hour sessions on 12 consecutive days in standard operant conditioning chambers. Rats were trained to press a lever on a fixed ratio 1 (FR-1) schedule for an infusion of heroin-hydrochloride (.1 mg/infusion for day 1–2, .05 mg/infusion for day 3–4, .025 mg/infusion for day 5–12; NIDA, Rockville, Maryland) over 4 sec, which initiated a 20-sec time-out period signaled by a light cue located above the lever (7). After the last session of self-administration, the rats were divided into two groups. One group was pretreated daily with NAC (100 mg/kg, IP; Sigma, St. Louis, Missouri) for 15 consecutive days, the other group with saline. Animals were administered saline or NAC 2.5 hours before extinction training or the first cue and heroin reinstatement trials. Thus, all subjects were injected daily with NAC or saline for 15 days. The 2.5-hour pretreatment period was chosen because this is the time required for NAC treatment to elevate extracellular glutamate in the brain (3).

During 15 days of NAC or saline treatment, the rats first underwent 7 ($n = 6$) or 10 daily ($n = 41$) 3-hour extinction sessions in the operant chamber without the light cue, during which responses on either lever had no programmed consequences (the six animals with 7 extinction days received NAC or saline in the home cage an additional 3 days before beginning reinstatement trials). After this extinction training all rats were exposed to a cue-induced reinstatement trial during which active lever presses resulted in presentation of the light cue that was previously associated with heroin infusion (8). After the cue trial,

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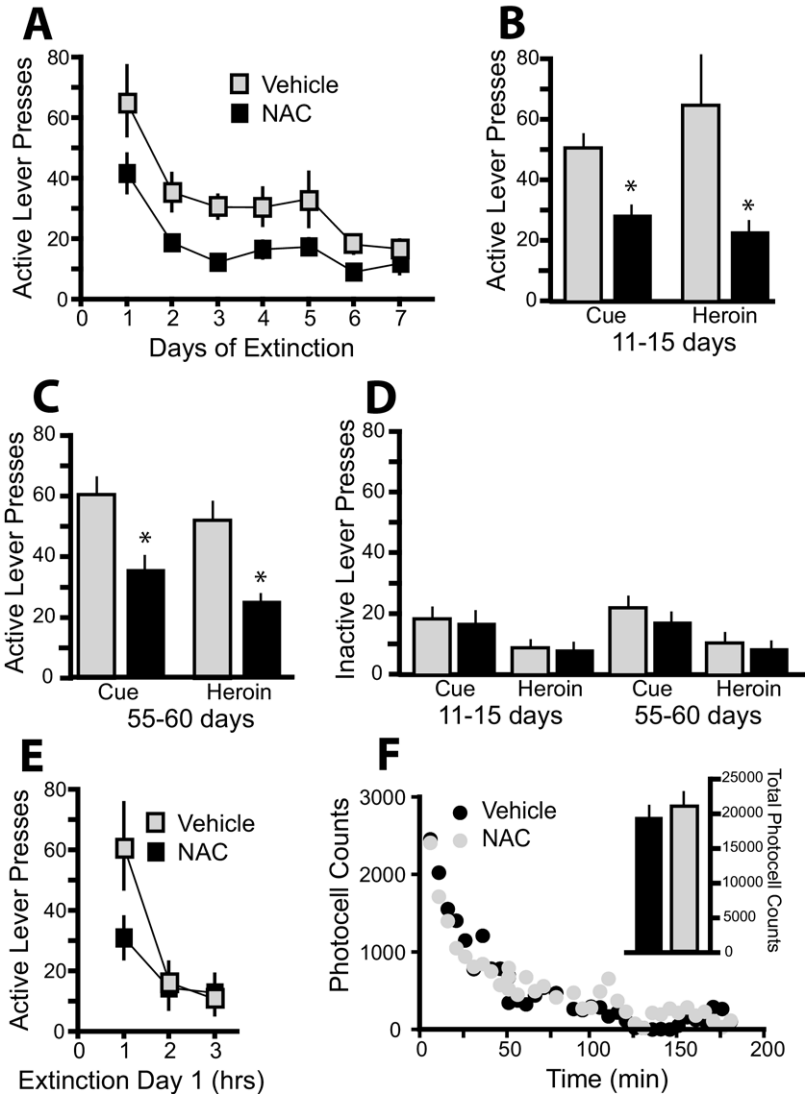

Figure 1. Daily N-acetylcysteine (NAC) administration facilitates extinction training and produces enduring inhibition of cue- and heroin-induced reinstatement. **(A)** NAC (100 mg/kg, IP) or vehicle was administered 2.5 hours before each 3-hour extinction training session. All animals were extinguished for 7 days (NAC, $n = 24$; Vehicle, $n = 23$), and for days 8–10 all but three rats in each group continued to receive extinction training. Regardless of the extinction training period, on days 1–10 all animals received respective pretreatments with NAC or vehicle. **(B)** Daily NAC and vehicle injections continued for 5 more days. On day 11 animals were presented with a light cue and on day 15 received an injection of heroin (.25 mg/kg, sc). **(C)** Animals were returned to the home cage for 40 days without any drug treatments. On day 55 they were exposed to light cues without any drug treatments. After an additional 4 days in the home cage, all animals were administered heroin without NAC or vehicle pretreatment. **(D)** Inactive lever presses during all reinstatement trials. All data are shown as mean ± SEM lever presses. **(E)** Data from one replication ($n = 10$ in each group) were analyzed in hourly intervals indicating that the effect of NAC treatment on day 1 extinction-responding occurred primarily in the 1st hour. **(F)** Photocell counts showing that chronic NAC did not alter behavioral responding in a novel open field environment compared with chronic vehicle treatment ($n = 7$ in both groups). *$p < .01$ comparing NAC with vehicle during the reinstatement trials.

daily NAC or vehicle administration continued in the home cage for 3 days. The next day, after NAC or vehicle, the rats were injected with heroin (.25 mg/kg, SC) in the operant chamber to induce reinstatement. After the two reinstatement tests, the rats were placed in the home cage for 40 days without NAC, vehicle, or heroin. Animals then underwent another cue and heroin reinstatement trial separated by 4 days but without NAC or vehicle pretreatment.

In a control experiment, rats were administered daily NAC (100 mg/kg, IP) or saline for 14 days; and 2.5 hours after the injection on day 15, the rats were placed into a photocell apparatus for 3 hours to measure the locomotor response to a novel open field environment (see [6] for details).

Results

After training to self-administer heroin, animals were placed into daily NAC ($n = 24$) or vehicle ($n = 23$) treatment groups to begin extinction training. The average number of active lever presses over the last 3 days of self-administration training was equal among the groups (NAC = 62 ± 17; vehicle = 58 ± 14, mean ± SEM). Figure 1A illustrates that NAC (100 mg/kg, IP) significantly augmented the extinction of active lever pressing compared with vehicle pretreated subjects; an effect most apparent during the first 5 days of extinction training (Figure 1A). A two-way analysis of variance with repeated measures over 7 days of extinction revealed a significant effect of NAC [$F(1,270) = 9.23$, $p = .004$] and withdrawal time [$F(6,270) = 16.71$, $p < .001$] but no treatment × time interaction. The 1st day of extinction was analyzed in hourly intervals, and the primary inhibitory effect of NAC on extinction lever pressing was during the 1st hour (Figure 1E). After 7 days of extinction training daily NAC and vehicle treatment groups showed equivalent levels of active lever pressing (NAC = 12 ± 4; vehicle 16 ± 4).

On day 11 animals were pretreated with NAC or vehicle and 2.5 hours later underwent a cue-induced reinstatement trial. Figure 1B illustrates that cue-induced reinstatement was significantly blunted by NAC [Student $t(45) = 3.089$, $p = .003$]. Animals were then administered NAC or vehicle daily in the home cage for 3 days. The next day (day 15) at 2.5 hours after NAC or vehicle pretreatment, all rats were injected with heroin (.25 mg/kg, sc) in the operant chamber. The NAC pretreated animals showed less heroin-induced reinstatement than vehicle pretreated subjects [Student $t(45) = 2.569$, $p = .014$]. Akin to the 1st day of extinction training, the

inhibitory effect of NAC was predominant in the 1st hour of the cue and heroin reinstatement trials (data not shown).

After the heroin reinstatement trial on day 15 (Figure 1B), animals were placed in the home cage for an additional 40 days without receiving injections of NAC, vehicle, or heroin. Figure 1C shows that after 40 days of abstinence, cue-induced reinstatement remained significantly blunted in animals that had been previously pretreated with daily NAC on days 1–15 [Student $t(39) = 3.075$, $p = .004$]. Similarly, after another 3 days in the home cage, animals were administered a heroin-priming injection in the operant chamber, and those previously pretreated with daily NAC showed significantly less reinstatement relative to control subjects [Student $t(39) = 3.733$, $p < .001$].

Figure 1D illustrates that inactive lever pressing during all reinstatement trials were equivalent between the NAC and vehicle treatment group. Figure 1F shows that chronic pretreatment with NAC did not alter the motor response elicited by placing a rat in an open field. Although this experiment indicates that chronic NAC was not inducing overt inhibition of motivated behavior, it remains possible that operant behaviors could be nonspecifically suppressed by NAC.

Discussion

These data show that, akin to the effects of acute NAC pretreatment in animals trained to self-administer cocaine (3,4), chronic pretreatment with NAC inhibits cue- and heroin-induced drug-seeking in the reinstatement model of relapse. Given the recent clinical trial showing that NAC was successful in reducing cue-induced drug desire in cocaine addicts (5), these data pose the possibility that NAC might also be successful in a similar trial in heroin addicts. Perhaps more significant is the finding that 40 days after discontinuing a 15-day NAC pretreatment regimen the capacity of cue or heroin to induce drug-seeking remained blunted. This presents the exciting possibility that repeated NAC treatment over a period of days might have a lasting inhibitory effect on relapse in addicts.

This initial study did not examine the mechanisms whereby NAC produced an enduring inhibition of reinstatement in heroin-trained animals. However, it is known that the inhibition of drug-seeking in cocaine-trained subjects arises, at least in part, from activation of cystine–glutamate exchange in the nucleus accumbens. This antiporter exchanges extracellular cystine for intracellular glutamate, is the rate limiting step in glutathione synthesis, and is downregulated after withdrawal from cocaine (3,9). The glutamate derived from cystine–glutamate exchange provides tone on presynaptic group II metabotropic glutamate receptors (mGluR2/3) that inhibit synaptic glutamate release probability (4,10). N-acetylcysteine is known to increase exchanger activity, thereby promoting glutathione synthesis (e.g., in treating acetaminophen overdose; 11), as well as increase glutamatergic tone on group II metabotropic glutamate autoreceptors (4,12). Consistent with this mechanism of action although not examined in the present study, it is possible that: 1) like cocaine training, heroin training is reducing cystine-glutamate exchange in the nucleus accumbens, and 2) repeated NAC treatment might cause an enduring restoration of exchanger function or other heroin-induced neuroadaptations in brain.

The enhancement of extinction training produced by NAC points to a potential added benefit of NAC to facilitate the extinction of drug-associated learning. Given the effect of NAC to stabilize synaptic glutamate transmission (4), these data are consistent with findings that a partial agonist at N-methyl d-aspartate (NMDA) receptors facilitates fear extinction training in phobic patients (13,14). However, further characterization is necessary to discern the behavioral mechanisms of the apparent facilitation of extinction training, especially given the fact that the inhibitory effect commences during the 1st hour of extinction training, pointing to a potential nonspecific effect. Although, it should be noted that the 1st day of extinction was the 1st day of NAC pretreatment, and it was previously shown that acute NAC administration did not alter food-primed reinstatement or rates of cocaine self-administration, indicating that nonspecific effects of NAC on behavioral responding are unlikely to account for the inhibition of day 1 extinction (3).

In conclusion, the present data provide a strong preclinical indication that NAC might have utility in treating heroin addiction. This is supported not only by the inhibition of drug-seeking induced by drug associated cues but also by the enduring inhibition of drug-seeking for over 1 month after discontinuing daily NAC treatment as well as the facilitation of extinction training.

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Supplementary material cited in this article is available online.

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